First Strand cDNA Synthesis Kit

(Cat. No.: S2GNM03j30001)

Description:

First Strand cDNA Synthesis Kit contains recombinant MMLV reverse transcriptase with improved thermostability and reduced RNase H activity. It is an easy-to-use kit for reliable cDNA synthesis, which is able to synthesize the first strand cDNA at 37~50°C.

This kit includes RNase Inhibitor, which effectively inhibits RNase A, RNase B, and RNase C activities, ensuring the integrity of RNA during the reverse transcription process. For added versatility, the product comes with both oligo $(dT)_{20}$ and random hexamers, allowing the synthesis of cDNA from poly(A) tailed mRNA and total RNA, respectively. With these features, the First Strand cDNA Synthesis Kit offers a reliable and flexible solution for your cDNA synthesis needs.

Kit Contents:

Contents	S2GNM03j30001 (100 rxns)
Reverse Transcriptase (200 U/µl)	100 µl x 1
RNase Inhibitor (20 U/µl)	100 µl x 1
5X RT Buffer (DTT)	500 µl x 1
dNTPs Mix (10 mM each)	200 µl x 1
Oligo $(dT)_{20}$ (50 µM)	100 µl x 1
Random Hexamers (100 µM)	100 µl x 1
DEPC-Treated H_2O	1 ml x 2

Storage:

The kit is stable for 24 months at -20°C.

Protocol:

1. After thawing, mix and briefly centrifuge the components of the kit, keep it on ice.
2. Add the following reagents into a PCR tube and keep it on ice.

Table 1. Reaction Setup - Denature (Mixture A)	
Components	Volume
RNA template	X µl (1ng~2 µg)
dNTP Mix (10 mM each)	1 µl
Primers* 50 µM Oligo $(dT)_{20}$ or 100 µM Random Hexamers or 10 µM Gene Specific Primers	1 µl
DEPC-Treated H_2O	to a final volume of 10 µl

Mix well and heated at 70°C for 5 minutes in advance, then incubated on ice bath at least 1 minute. Then, add other components according to the table.

Table 2. Reaction Setup - First strand cDNA buffer (Mixture B) per reaction (This Master Mix can be prepared before or during the denaturing step)	
Components	Volume
5X RT Buffer (DTT)	4 µl
DEPC-Treated H_2O	4 µl
RNase Inhibitor	1 µl
Reverse Transcriptase	1 µl
Final volume	**10 µl**

3. Mix the following reaction mixture, then incubate at (25°C for 10 minutes)* and 37-50°C for 50 minutes.

*For random hexamers, an additional 10 minutes of incubation at 25°C is suggested.

Table 3. Reaction Setup	
Components	Volume
Mixture A	10 µl
Mixture B	10 µl
Final volume	**20 µl**

4. For Termination：85°C/5 minutes, then keep at 4°C

5. (Optional step) For long-range RT-PCR reactions, it is recommended to add 1 µl RNase H into each reaction and heat at 37°C for 20 minutes.
6. Store cDNA at -20°C or for immediate PCR reaction

Recommended PCR Condition

Table 4. Reaction Setup	
Components	Volume
cDNA	2-10 µl
10X *Taq* Buffer	5 µl
Forward primer	0.1 – 0.5 µM
Reverse primer	0.1 – 0.5 µM
dNTPs	0.2 mM each
Taq DNA polymerase	0.25 µl (1.25 units)
H_2O	to a final volume of 50 µl
Final volume	**50 µl**

Recommended PCR Program

Table 5. Thermal Cycling Program			
Step	Temperature	Time	
Initial denaturation	94°C	2 mins	
Denaturation	94°C	30 sec	25-40 cycles
Annealing	50-68°C #	30 sec	
Extension	72°C	30 sec/kb	
Final Extension	72°C	1 min	

The optimal PCR conditions differ based on the thermodynamic properties of the primers.

◎ **Revision History** ◎

Description	Version	Date
Initial Release	S2GNM03j30001_Protocol_V1	Aug 2023